Information about bottom of iceberg of ammonia measurements in human plasma: Evaluation of uncertainty

Uncertainty of plasma ammonia measurement

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Abstract

Aim: Uncertainty of measurement (UM) defines the distribution of quantity values attributed to a measurand. The clinician assesses how much of the reported test result reflects its true value. UM for plasma ammonia was estimated according to the International Organization for Standardizairton (IOS ISO is actually the same organization as IOS. 15189) requirements in our laboratory. Material and Method: Plasma Ammonia of UM was calculated in accordance with The Guide to The Expression of Uncertainty of Measurement (GUM) and European Analytical Chemistry (EURACHEM) principles. Laboratory reproducibility was estimated with internal quality control (IQC), while external quality assessment (EQA) results were used to estimate bias and bias uncertainty. Uncertainity of reagents and calibrators was similarly determined. Using this data, including standard uncertainty, is combined with expanded uncertainty, and was also determined for ammonia. Results: The expanded uncertainty (k = 2) of ammonia was determined as 14.72 % (95% confidence interval). Discussions: Ammonia is a sensitive test which can be easily affected by preanalytcal errors; thus, it is important that the report is comprised of all analytical uncertainity factors for ammonia. Reporting UM for ammonia answered some questions (e.g., 'How effective are the analytical sources in the analysis of ammonia?' and 'What is the true value of blood ammonia?'). This study may be a prime UM example of ammonia's function in terms of the literature.

Keywords

Plasma Ammonia; Uncertainty; Internal Quality Control; External Quality Control; Within-Laboratory Reproducibility

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Introduction

The uncertainty of measurement (UM) is the distribution of the values that could reasonably be based on the measurand as defined by International Vocabulary of Metrology (VIM) and Guide to the Expression of Uncertainty of Measurement (GUM) as mentioned Joint Committee for Guides in Metrology (JCGM) 2012 and 2008 [1].

If UM is accompanied with measurand in the laboratory report, clinicians' will have more reliable information about the expression of results. By this report format which consists of variables such as reagents, calibrator within-laboratory reproducibility, external quality control results, the clinician gains an insight to the quality of the measurement.

Additionally, It is useful for comparing the quality of metrology among accredited clinical laboratories and helps in the interpretation of measurement results, especially when they are close to critical values. ISO/ IEC 17025:1999, ISO 15189 defines some standard requirements of international accreditation for estimation of the uncertainty of a test result. The analysis methods suitable for these procedures are mentioned in GUM, published by ISO. UM may be estimated by two different approaches: the bottom-up approach, and the top-down approach as mentioned JCGM [2]. The bottom-up approach suggested in the GUM is based on a wide investigation of the measurement, in which each potential resource of uncertainty is recognized, quantified and integrated to create a whole estimate of the uncertainty of the result with the use of statistical distribution rules. This model has been confirmed by metrology institutions and suppliers of reference materials. Accredited laboratories use these reference measurement procedures. UM mainly reflects the effect of bias and within-laboratory reproducibility (or precision) [3, 4, 5].

Bias is obtained by using appropriate reference material or external quality control material. It is expressed as systematic variables. While within reproducibility is expressed as random variables by using internal quality control materials in laboratories as mentioned Eurolab 2007.

Ammonia is normally produced by deamination of amino acids and metabolized to urea by hepatocytes. Ammonia is toxic if it accumulates in the body. Some diseases such as urea cycle defects, hepatic dysfunction cause blood ammonia elevation [6]. It is essential to give reliable results in diseases associated high ammonia levels. In this paper, plasma ammonia UM is calculated using reagent, calibrator, internal quality control (IQC), and external quality control (EQC) assessment data in order to obtain high-quality patient test results.

Material and Method

In this study, data from the reagent, calibrator, IQC and EQC samples were used. No ethical committee approval is required since patient data were not used for the samples included in this study. All sources of uncertainty for plasma ammonia is determined by fishbone diagram in Figure 1. Values of uncertainity of external quality assessment data are given in Table 1. For the sources and values of uncertainty of plasma ammonia see Table 2.

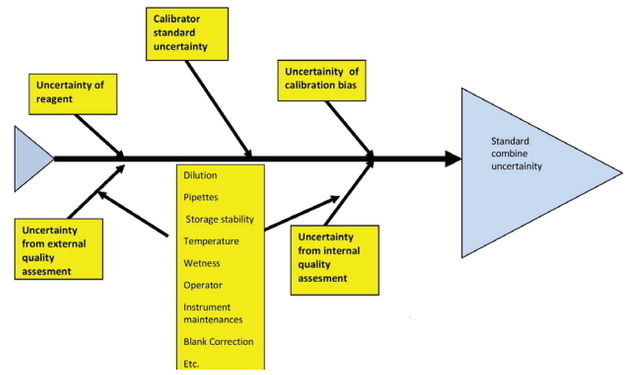

Figure 1. The fishbone diagram bottom-up approach to plasma ammonia uncertainty evaluation according to the Guide to the xpression of uncertainty in measurement.

Table 1. Uncertainity of external quality assesment data

Samples (n)	Ammonia (Bias%)	Ammonia Bias $_{EQA}{}^{2}$
1	0.0142	0.00020164
2	0.0520	0.002704
3	0.0568	0.00322624
4	0.0417	0.00173889
5	0.0057	0.0003249
Σ Bias $_{EQA}{}^{2}$		0.00790326
$U_{EQA}{}^{2}=\Sigma(Bias_{EQA})^{2}/n$		0.001580652

Table 2. The sources and values uncertainitiy of ammonia

Uncertainity sources for ammonia	Relative Standard uncertainity
reagent	0.02900
calibrator	0.052807
calibration bias	0.012701
Within laboratory reproducibility (For IQC normal level)	0.009060
Within laboratory reproducibility (For IQC high level)	0.006400

Measurement of ammonia

Ammonia was measured using a glutamate dehydrogenase enzymatic method by infinity ™ Ammonia reagent with Beckman Coulter AU 5800 Biochemistry autoanalyzer (USA); Lot number OSR61154 for reagents and calibrator. Sample collection, transportation, and analysis were carried on according to the manufacturer's instructions. In this method α-ketoglutarate is converted to glutamate and NAD produced is proportional to the ammonia concentration. Reference intervals were adopted from published manufacturer ranges and verified using normal volunteers as 27 - 90 µg/dL. The analytical measuring range of the assay is 17 - 1020 µg/dL.

Internal quality control material

Ammonia/Alcohol control for normal level (range, 41–109 µg/dL, lot number M703701, level 1), lot number M703702 for high level (range, 194-262 µg/dL, lot number M703702, level 2) were used as internal quality control (IQC) samples (UniCel DxC Synchron Systems, Beckman Coulter). These were measured at two concentration levels at each run. Data from IQC were then obtained for the consecutive fifty-six result for same lot number, so as to ensure that potential variations due to calibration

by different operators, reagents, product batch numbers and routine maintenance, were accounted. Data from every rejected run were omitted.

External quality control material

Bio-rad Laboratories, as external quality assessment (EQA) provider, sends out EQA survey samples in Ethanol/Ammonia Program twelve times per year. Bio–Rad Laboratories is accredited by the American Association for Laboratory Accreditation (A2LA) to the ISO/IEC 17043:2010 standard (Biorad, California, USA). Laboratories measure the samples and upload the results to the Bio-rad website. After submission of results, statistical analysis was performed according to the ISO 17043:2010 guidelines. The laboratories received EQA report, which included the results itself and the consensus values from laboratories using the same type of assay. The six sequent period EQA data sets of ammonia analyte were used for the evaluation of UM. Beckman Coulter AU 5800 biochemistry autoanalyzer has been serving since September 2017 in our laboratory. Therefore, last five months data of EQA were investigated for this study.

Determination of uncertainty sources and calculation of uncertainty components

GUMand EURACHEM guidelines were used to calculate the measurement uncertainty of the ammonia test performed in our laboratory. The following sources that may lead to uncertainty for these tests have been reviewed [3]:

1. Calibrator and Calibration Uncertainty (Type A)(Group evaluated by statistical methods) U (calibrator): The standard uncertainty (U (calibrator)) value was calculated by working of the calibrator 10 times in succession and finding the standard deviation. Calibrator worked 10 times consecutively for the same patient sample. Then standard deviation (SD) and standard uncertainty (SD / √n) were calculated with these results as follows:
U (calibrator) (Calibrator standard uncertainty)=(SD/√n)x(1/X_{mean})
n: number of repeated measurements

2. Uncertainty of Calibration Shift (Type A)(Group evaluated by statistical methods) (U (calibrator-bias)): The uncertainty from the calibration curve shift is found by dividing the maximum shift value by √3 "(rectangular distribution). The arithmetic mean of the single-level calibrator that was run 10 times in a row was taken. This mean value was subtracted from the target value to calculate the maximum shift value and the difference is divided by the target value. With the assumption of rectangular distribution, this quotient is divided by √3.

3. Uncertainty of Reproducibility (Type-A)(Group evaluated by statistical methods)(U_{Rw}): CV% for ammonia was calculated using fifty-six consecutive IQC material values of the same lot number.

4. Uncertainty of the certificate value of the reagent (Type B) (Group evaluated by non-statistical methods) (Ur): CV% values for within run and for total repeatability of the reagent are given by the manufacturer. The greatest CV% of the reagent provides us with the highest uncertainty estimate. This value is recorded as Ur (reagent) when the measurement uncertainty is calculated.

5. Uncertainty by external quality control performance data (U_{EQA}): Interlaboratory bias were calculated using EAQS data. Bias is calculated as the deviation rate of the laboratory test result from the test result averages [= (Test result - Compared group mean) / Compared group mean]. The calculated EQA report biases are used to calculate the uncertainty by external quality control value as shown in the formula below.
$U_{EQA} = (\Sigma(Bias_{EQA})^2/n)^{1/2}$
n = Number of EQA
U_{EQA}: Uncertainty by External Quality Control

6. Calculation of combined standard uncertainty: (Uc)

Combined standard uncertainty was calculated using uncertainties of all sources.

7. Expanded uncertainty (Ue)

According to the characteristics and requirements of the medical laboratory, the coverage factor (k) 2 produces an interval having a level of confidence of approximately 95 %.
Ue = k×Uc

Statistical analysis

For statistical purposes, descriptive statistics95% confidence interval calculation were used.

Results

Calculation of Uncertainty Components in Ammonia Measurement

1. Calibrator standard uncertainty: (U (calibrator)

The calibrator with a concentration of 100 µg / dL was run 10 times (n = 10) consecutively. The mean for these was found to be 102.2 with a standard deviation of 17,06667.
The standard uncertainty was calculated as 5.396956.
U(calibrator) = (17.06667/√10) x (1/102.2) = 0.052807 was found for ammonia.

2. Uncertainity of calibration bias: U(calibrator bias)

When calibrator was run for the same patient for 10 times, maximum ammonia shift value is obtained at 2.2%.
Calibrator value = 100 µg / dL
Mean = 102.2
Calibrator bias value = (100-102.2)/100 = 0.022
U(calibrator bias)(Uncertainity of calibrator bias) = 0.022/ √3 = 0.012701

3. Within-laboratory reproducibility: U(Rw)

CV% = 6.78% was found for normal IQC level of ammonia.
CV = 0.0678
U(R-1) = 0.0678/√56 = 0.009060
CV% = 4.77% was found for high IQC level of ammonia.
CV = 0.0477
U(R-2) = 0.0477/√56 = 0.006400
Uw = U(R-1) + U(R-2)/2 = 0.009060+0.006400/2 = 0.007730

4. Uncertainty of reagent: U(reagent)

The highest CV%value was reported as 5 for ammonia by the manufacturer.

U(reagent) = 0.05/√3 = 0.028867

5. Uncertainty by external quality assessment (EQA) performance data: (UEQA)

Serum ammonia level was determined according to the results of the last 5 months analysis of the EQAS program in which the laboratory was included in the previous year for uncertainty from EQCperformance data (Table 1).

U_{EQA}^2 = 0.039757

6. Calculation of combined standard uncertainty: (Uc)

$Uc = \sqrt{[U_{(calibrator)}^2 + U_{(calibrator\text{-}bias)}^2 + U_{(Rw)}^2 + U_{(reagent)}^2 + U_{(EQA)}^2]}$

$Uc = \sqrt{[(0.028867)^2 + (0.052807)^2 + (0.012701)^2 + (0.007730)^2 + (0.039757)^2]}$

Uc = √[0.000833+ 0.002788+0.000161+0.000059+0.001580]

Uc = √0.005421 = 0.073627

Uc = 7.36 % for ammonia

7. Expanded uncertainty (Ue)

Ue = 2x7.36 = 14.72% for ammonia.

Discussion

The aim of this study was to calculate the UM for plasma ammonia using the procedures recommended by the GUM and EURACHEM guidelines. The within-laboratory reproducibility was estimated from consecutive fifty-six IQC data of the same lot numbers at two different levels. Bias was determined according to interlaboratory comparisons using EQA results. In this manner, the effects of some systematic uncertainty factors were evaluated.

Increased levels of plasma ammonia pass through the blood-brain barrier and affect adversely the brain tissue. This can cause deterioration of cognitive functions, which can present in various process. Finally, considerable cerebral damage occured [7]. Because of elevated levels, plasma ammonia is associated with a high morbidity and mortality, effective treatment for the reduction of plasma ammonia levels are important in the management of these diseases for clinicians. The diagnosis of this state is important because it is often treatable [8]. However, preanalytical factors such as inappropriate transport and storage, procedure delay can cause false positive ammonia levels [9, 10]. Therefore, some important preanalytical procedures must be followed; samples should be kept cold immediately after sampling, centrifuged, aliquoted, and analyzed within 15-30 minutes for accurate analysis of ammonia. If preanalytical factors are under control, reported UM reflects analytical sources effect.

The determined or standardized percent of UM are not yet adviced for plasma ammonia levels, in clinical laboratories. For this reason, interlaboratory comparisons are not possible. On the other hand, for accreditation of a laboratory UM is a prerequisite but in the future, there may be an expectation of definite percents.

Although in the present literature most of the factors causing sources of preanalytical error in ammonia measurement have been described [9, 10]; there is no previous study on UM for plasma ammonia.

Ammonia obtained from the catabolism of amino acids and the action of intestinal bacteria on the diet protein is converted to urea in liver hepatocytes and rendered non-toxic [11]. Under normal conditions, the concentration of ammonia in the circulation remains low, usually below 85 µg / dL (50 µmol / L) in Beckmann Coulter reagent insert, reference range was 15-45 µg / dL in adult, reference range is different from newborn to two years old [12].

Guidelines for detection and management of hyperammonaemia suggest that levels of ammonia up to 280 µg / dL (200 µmol/L) are associated with acquired conditions such as sepsis, chemotherapy or liver dysfunction, while ammonia levels higher than 280 µg / dL indicate metabolic disorders as mentioned guidelines for hyperammonemia in United Kingdom national metabolic biochemistry network.

The ammonia levels of the internal quality control sample used in our laboratory were also appropriately selected for diagnostic purposes.

According to our findings, an ammonia analyze result of 81.6 µg / dL (normal IQC level) by our laboratory means a level interval between 69.59 µg / dL and 93.61 µg / dL with a confidence interval of 95%. For ammonia analyze result of 245.7 µg / dL (high IQC level) reported our laboratory, the result actually lies somewhere between 209.54 µg / dL and 281.86 µg / dL, with a 95% confidence interval. If the clinician had the 245.7 µg / dL value only, the diagnosis would possibly be "metabolic disorder", but with the confidence interval values the clinician's decision will change, and may decide further evaluation. Furthermore, if this patient's plasma ammonia level measurements are repeated at our laboratory or at another laboratory with the same UM methodology, the clinician will have the opportunity to compare the results.

The evaluation of UM interval may be assessed by total allowable error (TEa) based on biological variation components [13] because there is no any source about UM interval limits for ammonia.

American Association of Bioanalysts (AAB) advised TEa for serum ammonia as ± 14 µg/dL (10 µmol/L) or ± 5%. The Royal College of Pathologists of Australasia (RCPA) advised up to ± 20% for ammonia (Lower Goal ± 5 % ≤ 70 µg/dL (50 µmol/L), Upper Goal ± 20% >70 µg/dL) as total allowable error (TEa) while clinical laboratory improvement amendments (CLIA) does not advise any ratio for ammonia.

Our laboratory UM for ammonia was 14.72% and this value is in accordance with TEa recommended by RCPA.

The TEa defines the upper and lower limits and it formulates the event over the limits of systematic error and random error. By this formulation some of the results from external quality control evaluation results are used for systematic error evaluation and the CVs from internal quality control evaluation results are used for random error evaluation. In this sense, there are similarities between TEa and UM calculation. In addition to the CV from the EQC, which comes from the internal quality control results, the maximum CV value of the reagent given by the reagent manufacturer is added to the calculation of the uncertainty probabilities that may arise from the calibrator shift

while calculating the UM. In addition to informing the clinician that the laboratory contribution of the UM reflects a certain interval of the results, it also leads to the revealing of sources of uncertainty. It is also an important advantage which allows to compare between laboratories using the same UM calculation method. However, since UM computation involves more complex mathematical formulas, the use at non-accredited laboratories is not common. Formulas can be integrated into the laboratory information system for each test. These models have differences, but these should not be over-emphasized. TEa e.g. defines a region around the reference ("true") value where measured analytical results can be found with a defined probability. UM defines a region around the measured analytical result where the "true" value can be found with a defined probability. The similarity between both models is, that they both express the reliability of the test result, however from a different perspective [14].

There is an uncertainty for each result presented by a laboratory. It may be ascribed to a number of small variations originating at any phase of the analytical process if preanalytical process is followed. It is important to understand that uncertainty is not the same as an error. An error means that there is a difference between a measured value and the true value caused by an unknown factor, whereas uncertainty is an acceptable interval (UM) within which a result can fall. If laboratory experts need some constrict for this interval, they may intervene sources of UM such as bias or use another reagent.

A recent study showed that the reference change values (RCV) of (using the within-subject and between-subject biological variation) ammonia was found to be 43.7 % for healthy subjects in fresh samples [15]. If we evaluate our laboratory UM according to RCV for ammonia, 14.72% ratio is lower than this ratio.

UM is useful for a number of reasons; it gives information about the quality of the measurements and is useful for comparing the metrological quality of several clinical laboratories (among accredited clinical laboratories, provided that it is calculated in the same way), and helps interpretation of measurement results, especially when close to critical disease -defining values. In fact, when comparing a result with a decision limit we can give clear information to the clinician only if the limit is not included within the uncertainty around the result. Thus, there is no doubt that the concept is valuable.

Our UM results are below the levels of RCPA ammonia values. There is no any reference study for ammonia UM.

In practice, by estimating and reporting uncertainty, the laboratory can indicate the quality and reliability of the reported result. Then, this information can be used by clinicians to compare a result to a cut-off limit or to a previous result from a sample for the same patient. Such information can be used by a clinician to determine whether the difference between two results is negligible due to uncertainty or significant due to a change in the condition of the patient. Without an estimate of UM, comparisons of previous results or reference values are not satisfactory.

In conclusion, this paper is the first in the literature that suggest an interval calculation for UM of plasma ammonia. UM is important for the clinician in the management of diseases with elevated ammonia levels. The addition of UM for ammonia to laboratory report enriches the reliability of the results as advised and required as a quality indicator.

Scientific Responsibility Statement

The author declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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